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Stability of *Methanobrevibacter smithii* Populations in the Microbial Flora Excreted from the Human Large Bowel

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Total anaerobic bacteria and *Methanobrevibacter smithii* populations were enumerated in fecal specimens from two individuals over 10- and 13-month periods. The ratio of *M. smithii* to total anaerobic count varied between the individuals, but it was a relatively constant proportion of the large-bowel microbial flora within each individual. Neither a barium enema examination of one subject nor a radical change in the diet of the other had any long-term effect on the methanogen populations.

Methane is produced by bacteria in the large intestine of some humans (1). About 20% of the methane is absorbed into the blood and excreted via the lungs (2). Using breath-methane analysis, Bond et al. (1) have shown that one-third of the adult human population excretes methane. The amount of excreted methane varies over a 2-log range among individuals, but for a particular individual it remains relatively constant (1).

We have enumerated methanogens in human feces by using a plating medium containing clindamycin and cephalothin and concluded that *Methanobrevibacter smithii* is the most numerous methanogen in feces from individuals who harbor methanogens in the large bowel (4). The concentrations of methanogens in individual subjects varied over a 3.5-log range from ca. 10^7 to 10^{10}/g (dry weight).

To determine the variation of the methanogen concentration of an individual over an extended time period, we used this same antibiotic plating medium to enumerate the methanogens. In a long-term study of the stability of *M. smithii* in the feces of two methane-positive individuals, the methanogen concentrations in the two individuals remained relatively constant over the time courses examined (10 and 13 months). Neither a barium enema examination of one subject nor a radical change in the diet of the other had any effect on the methanogenic populations.

The Hungate technique, modified for serum bottles, was used to prepare media and grow cultures (3). All incubations were at 37°C, and liquid cultures were agitated by shaking or rolling. Two methanogenic individuals who had participated in an earlier study (4; subjects B and D) agreed to provide the monthly fecal specimens.

Freshly voided feces were collected in clean cardboard cups. Samples were processed immediately or refrigerated and processed within 2 h of defecation. Portions of fresh feces (ca. 2 g [wet weight]) were accurately weighed and dried in an oven at 180°C for 24 h. Samples were transferred to a desiccator containing Drierite (W. A. Hammond Drierite Co., Xenia, Ohio) and dried to constant weight.

A 10^{-1} dilution of every specimen from subject B and of the first four specimens from subject D were prepared, as described previously, in anaerobic dilution solution containing glass beads (4). The later specimens from subject D were mixed in a Stomacher Lab-Blender 80 (Tekmar Co., Cincinnati, Ohio) for 30 s, and a 10^{-1} dilution was prepared in anaerobic dilution solution and mixed in the Stomacher for 2 min. The 10^{-1} dilution was transferred to a sterile serum bottle, which was gassed with 100% CO_2, stoppered, and crimped. A separate study showed that portions of the same fecal specimen processed by the glass bead or Stomacher method gave essentially identical results.

For total anaerobe counts, 0.1 ml of each appropriate dilution was enumerated in duplicate on a roll tube of complex agar medium containing 10% rumen fluid and additional NH_4Cl under 100% CO_2, as previously described (4). Methanogens were enumerated in duplicate in the same medium containing cephalothin, clindamycin, and 80% H_2–20% CO_2 (4). Roll tube colonies were counted at 7, 8, or 9 days; colony counts did not increase thereafter. Portions of the headspace of antibiotic roll tubes containing colonies were analyzed for CH_4 as previously described (4). Serum bottles containing 10^{-1} dilutions were aseptically regassed and pressurized to 2 atm (202.6 kPa) with 80% H_2–20% CO_2 and incubated. After ca. 24 h, 0.5-ml portions of the headspace were analyzed for

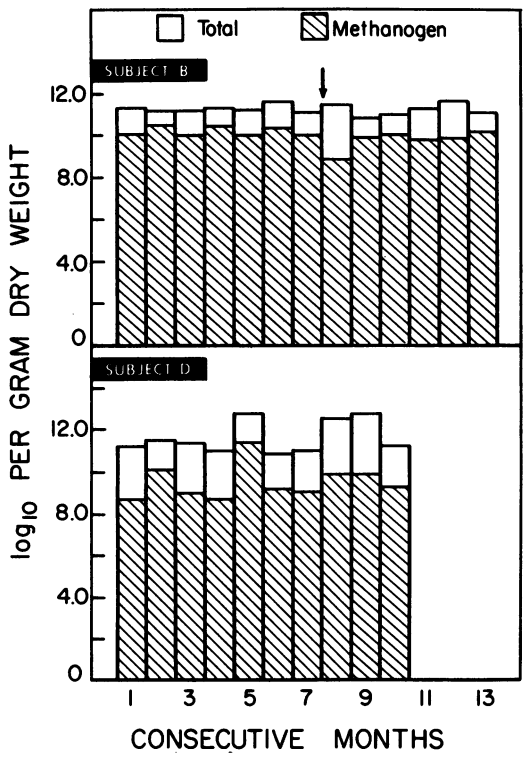

FIG. 1. Total viable anaerobes and *M. smithii* (methanogen) in monthly fecal specimens from two methanogenic subjects. Subject B had a barium enema 8 days before the month 8 sample (arrow).

CH_4 by gas chromatography (4).

The results of the enumeration of total anaerobic bacteria and methanogens for the two subjects are given in Fig. 1. The suspension of every fecal specimen from each individual produced CH_4 and had Factor 420-fluorescing coccobacillary rods. Methods for determining methanogenesis by suspension and for detecting the fluorescent cells were as previously described (4).

Subject B was a 32-year-old, healthy man who consumed a typical western diet throughout the study. He was initially identified as harboring high concentrations of *M. smithii*, ca. 10^{10}/g (dry weight) (4). Eight days before collection of the month 8 specimen, this individual underwent a barium X-ray series. (He was subsequently found to have no clinical symptoms of disease.) In the month 8 specimen, although the total anaerobe count was similar to that of previous specimens, the methanogens were only ca. 0.2% of the usual total count. By month 9 the methanogen population had returned to the earlier concentration. The average total anaerobe count for 13 months was log 11.25 ± 0.23/g (dry weight). The average concentration of methanogens (excluding month 8) was log 10.10 ± 0.21/g (dry weight), or 7.1% of the total anaerobes.

Subject D was a 28-year-old woman. She was initially found to harbor moderate concentrations of *M. smithii*, ca. 10^9/g (dry weight) (4). Two months into the study she began a high-protein, low-carbohydrate (≤50 g/day) diet of 600 calories per day. After 4 months the diet was modified, the carbohydrate intake remained at ≤50 g/day, but the caloric intake was increased by 100 calories per day each week up to a final daily intake of 2,000 calories. During the course of this diet the subject lost 57 lb. The total anaerobe counts for this subject varied somewhat more than those for subject B (Fig. 1). During month 5 the total anaerobe and methanogen counts were extremely high compared with those in other fecal specimens obtained from this individual. Excluding month 5, the total anaerobe count was log 11.54 ± 0.66/g (dry weight), and the methanogen count was log 9.31 ± 0.66/g (dry weight) (0.6% of the total anaerobe population).

Although methanogens were not isolated from all plated specimens, the predominant Factor-420 fluorescent morphotype in all fecal suspensions and the morphology of the colonies in the antibiotic medium were characteristic of *M. smithii*. Pure cultures of a methanogen were isolated from the month 12 specimen of subject B and from the month 9 specimen of subject D. Both isolates were morphologically and immunologically identical to *M. smithii*, confirming our previous report that *M. smithii* is the most numerous methanogen in the large bowel of these two individuals (4). The results indicate that although the ratio of *M. smithii* to total anaerobe population may vary among individuals, it comprises a relatively constant proportion of the large bowel microbial flora within each individual.

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